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Xu, Helan; Canisag, Hazal; Mu, Bingnan; and Yang, Yiqi, "Robust and Flexible Films from 100% Starch Cross-Linked by Biobased Disaccharide Derivative" (2015). *Biological Systems Engineering: Papers and Publications*. 549.
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ACS Sustainable Chemistry & Engineering

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Robust and Flexible Films from 100% Starch Cross-Linked by Biobased Disaccharide Derivative

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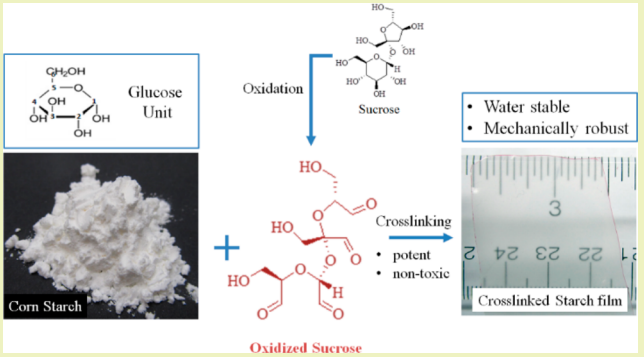
ABSTRACT: In this research, oxidized sucrose, a novel aldehyde-based green cross-linker, endowed starch films with substantial improvement in both tensile strength and elongation, whereas many other cross-linkers did not. Starch films are usually weak, brittle, and highly moisture sensitive, and thus have restricted industrial applications. Cross-linking is one of the most common methods to tackle these problems. However, most of the available cross-linkers are either toxic, expensive, or with low cross-linking efficiencies. Oxidized sucrose is a green cross-linker with multiple aldehyde groups per molecule to cross-link starch molecules via forming hemiacetals/acetals. The starch films cross-linked with oxidized sucrose had tensile strength and breaking elongation of 23 MPa and 60%, respectively, exceeding the cross-linking results of many other cross-linkers. Oxidized sucrose cross-linking also substantially increased the stability of starch films in both water and formic acid. With activation energy as low as 33.22 kJ mol^{-1}, the cross-linking, a pseudo-first-order reaction, could occur readily. Mild cross-linking using oxidized sucrose might provide an alternative to promote industrialization of starch-based products.



KEYWORDS: *Lowly toxic cross-linker, Starch, Oxidized sucrose, Biobased aldehyde, Flexible film*

INTRODUCTION

Because of depletion of petroleum resources and environmental issues associated with petroleum-based synthetic materials, bioplastics from renewable and degradable biomass resources arouse much interest in both academic and industrial areas. In addition to being capable of replacing synthetic polymers in various applications,[1] about 50% of bioplastics currently in use are derived from starch due to its low cost, diverse and abundant resources, and easy processability.[2]

However, moisture sensitivity and brittleness of starch-derived products restricted applications of starch in many industrial areas. To cope with these problems, physical and chemical methods were used to develop starch-derived products. Blending with synthetic polymers, such as polyethylene,[3] styrene−butadiene latex,[4] and poly(vinyl alcohol) (PVA),[5] was employed to strengthen the starch films and other starch-derived products. Incorporation of nanoscale fillers was also found capable of increasing tensile strength of starch films while considerably reducing their elongation.[6] However, nonbiodegradability of some incorporated substrates, poor compatibility between starch and synthetic polymers, and limitedly improved water stability, remained obstacles for development of products from starch blends.

Chemical cross-linking that induces formation of networks of molecules could also enhance the performance properties of starch products. Aldehydes might be the most frequently used chemicals for starch cross-linking due to their high reaction efficiency. Glutaraldehyde[7] and formaldehyde[8] are examples of aldehydes that are most widely used in cross-linking of macromolecules, including proteins and polysaccharides.[9,10] These highly reactive small-molecule aldehydes showed adequate enhancement in both mechanical performances and stability. Nevertheless, glutaraldehyde and formaldehyde are well-known for their environmental hazard and cytotoxicity,[11] and thus require specific equipment or procedures for their use in large-scale industrial and biomedical applications. In addition, epichlorohydrin was also used to cross-link starch.[12] However, upon contacting with water, epichlorohydrin could be readily hydrolyzed into 3-chloropropane-1,2-diol, which is a carcinogen.[13] Highly toxic phosphorus oxychloride was also used to cross-link starch.[14]

Received: April 27, 2015
Revised: September 28, 2015
Published: October 8, 2015

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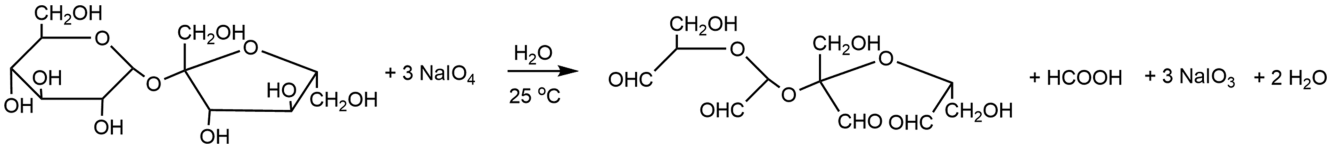
DOI: 10.1021/acssuschemeng.5b00353
ACS Sustainable Chem. Eng. 2015, 3, 2631−2639

Scheme 1. Oxidation of Sucrose into Polyaldehyde



Currently, nontoxic cross-linking methods are in high demand,[15] as most traditional cross-linkers have toxicity issues associated with their high reaction efficiencies. However, most of the cross-linkers lacked capability to improve tensile strength and elongation at the same time. Citric acid, an inexpensive and nontoxic natural poly(carboxylic acid), was applied to cross-link starch. The obtained starch films showed improved mechanical properties and prolonged water stability.[16] However, the percent elongation of citric acid cross-linked starch films was only up to 30%, indicating their limited extensibility. In additions, photo-cross-linking was an environmentally benign cross-linking approach, however, the treated starch films showed tensile strength even less than 10 MPa.[17]

Oxidized sucrose could be a new option as a potent biobased cross-linker. Saccharides were oxidized to polar aldehydes or polymeric aldehydes for their low toxicity and potentially high reaction efficiency. Oxidized polysaccharides, such as dialdehyde starch[18] and oxidized dextran[19] with proved low-toxicity, were used to cross-link proteins, such as soy protein[20] and egg white.[21] However, the cross-linking effects were unsatisfactory, indicated by the limited improvement in mechanical properties and water solubility. There are abundant aldehyde groups in each dialdehyde starch molecule. However, the starch molecules have degrees of polymerization ranging from 100 to 1000. The bulky backbones prevented the dialdehyde starch to fully participate into the cross-linking reaction with substrates, leading to insufficient cross-linking efficiency. On the other hand, oxidized sucrose could contain up to four aldehyde groups in one former disaccharide molecule available for reaction.[22] Oxidized sucrose already showed effectiveness in cross-linking zein, the corn protein.[23] The in vitro cell culture study indicated that the protein scaffolds cross-linked with oxidized sucrose were biocompatible.[23] Moreover, the polar backbones prevented it from being highly volatile, which was the main cause of environmental toxicity of formaldehyde and glutaraldehyde. To the best of our knowledge, there is no publication that reports on utilization of oxidized sucrose on cross-linking starch products.

In this study, starch film cross-linked with oxidized sucrose enhanced tensile strength and strain more efficiently than many other reported cross-linkers. The water and acid stability of starch film were also remarkably improved. In addition to FTIR and NMR for molecular characterizations, the cross-linking reaction was also investigated in terms of kinetics.

MATERIALS AND METHODS

Materials. Corn starch was purchased from Cargill, Minneapolis, MN. Formic acid and sulfuric acid (>98%) were purchased from EMD Chemicals Inc. Gibbstown, NJ. Sucrose, sodium periodate (>98%), and barium dichloride were purchased from Johnson Matthey Company, Ward Hill, MA. All the other chemicals were purchased from VWR International, Radnor, PA.

Preparation of Oxidized Sucrose. Oxidized sucrose was obtained via periodate cleavage. About 6.6 g of sucrose and 12.9 g of sodium periodate were dissolved in 200 mL of distilled water. After the solution was stirred at room temperature for 26 h,[24] about 7 g of barium dichloride was added and the mixture was stirred at 5 °C for 1 h to allow complete precipitation. The mixture was filtrated to obtain the liquid containing polyaldehyde derivatives of sucrose. The oxidized sucrose with concentration of around 6 wt % was stored at 5 °C for further use. The reaction was shown in the following Scheme 1.

Because the oxidized sucrose was a mixture, we estimated the molecular weight based on the average of the possible maximum weight (the weight of sucrose, 342.49 g mol^{-1}) and the possible minimum weight (the weight of oxidized sucrose with 4 aldehyde groups per molecule, 308.00 g mol^{-1}).

Solution Casting and Cross-Linking of Starch Films. About 3 wt % starch was dispersed in distilled water, heated at 90 °C for 30 min. For un-cross-linked control films, pure starch solution and starch solution with 15% glycerol (based on the weight of starch) were poured onto Teflon-coated plates. For cross-linked films, oxidized sucrose solution was diluted to disperse starch to make the final oxidized sucrose concentration 1, 3, 5, 8, 10, and 15 wt % based on the weight of starch. The mixture was heated at 90 °C for 30 min. Subsequently, required amounts of glycerol (5, 10, 15, 20, 30, and 40 wt % based on the weight of starch) were added into the solution, and then poured onto Teflon-coated plates. The cast films were allowed to air-dry for 72 h before peeled from the plates. The starch films were then treated in an air oven at various temperatures (140, 145, 150, 155, 160, and 165 °C) for various periods of time (3, 5, 8, 10, and 15 min). After heating, the films were balanced in a standard condition (21 °C and 65% relative humidity).

The oxidized sucrose solution had pH at around 2.5 due to the existence of the formic acid as a product from the oxidation. In the starch film, glycerol with high hygroscopicity could hold certain amount of moisture in the film. The presence of acid and moisture might facilitate the formation of acetals which usually requires acidic catalysis.

Characterization of Oxidized Sucrose and Reaction between Starch and Oxidized Sucrose. Fourier transform infrared (FTIR) spectra were collected from sucrose and oxidized sucrose on an attenuated total reflectance ATR spectrophotometer (Nicolet 380: Thermo-Fisher, Waltham, MA) to determine the structural difference after periodate cleavage. The samples were placed on a germanium plate and 128 scans from 500 to 4000 cm^{-1} were collected for each sample.

^{1}H NMR analyses were done to illustrate the structural differences of sucrose/oxidized sucrose and non-cross-linked films/cross-linked films. For oxidized sucrose, the solvent was evaporated and the resulting sample was dissolved in D_2O. The starch samples were dried and were dissolved in deuterated DMSO. Only soluble portion of cross-linked starch film in DMSO was collected for NMR analysis. Only soluble portion was used for NMR scanning. For cross-linked starch, more scans were taken until good signal-to-noise ratio was obtained. Spectra were taken on a Bruker AVANCE DRX 700 MHz NMR spectrometer (Bruker, Billerica, MA).

Static Mechanical Properties of Starch Films. Tensile properties of starch films were determined on an MTS tensile tester (Eden Prairie, MN) according to ASTM Standard D 882-02. Before testing, all samples were conditioned for at least 48 h in standard condition. Wet tensile properties were determined after conditioning the films at 21 °C and 90% relative humidity in a conditioning chamber (Caron, Marietta, OH) for 48 h. The effects of weight ratio of glycerol to starch, weight ratio of oxidized sucrose to starch, time, and temperature on cross-linking were studied.

Dynamic Mechanical Analysis (DMA). To determine the effect of cross-linking on the dynamic thermo-mechanical properties of

DOI: 10.1021/acssuschemeng.5b00353
ACS Sustainable Chem. Eng. 2015, 3, 2631−2639

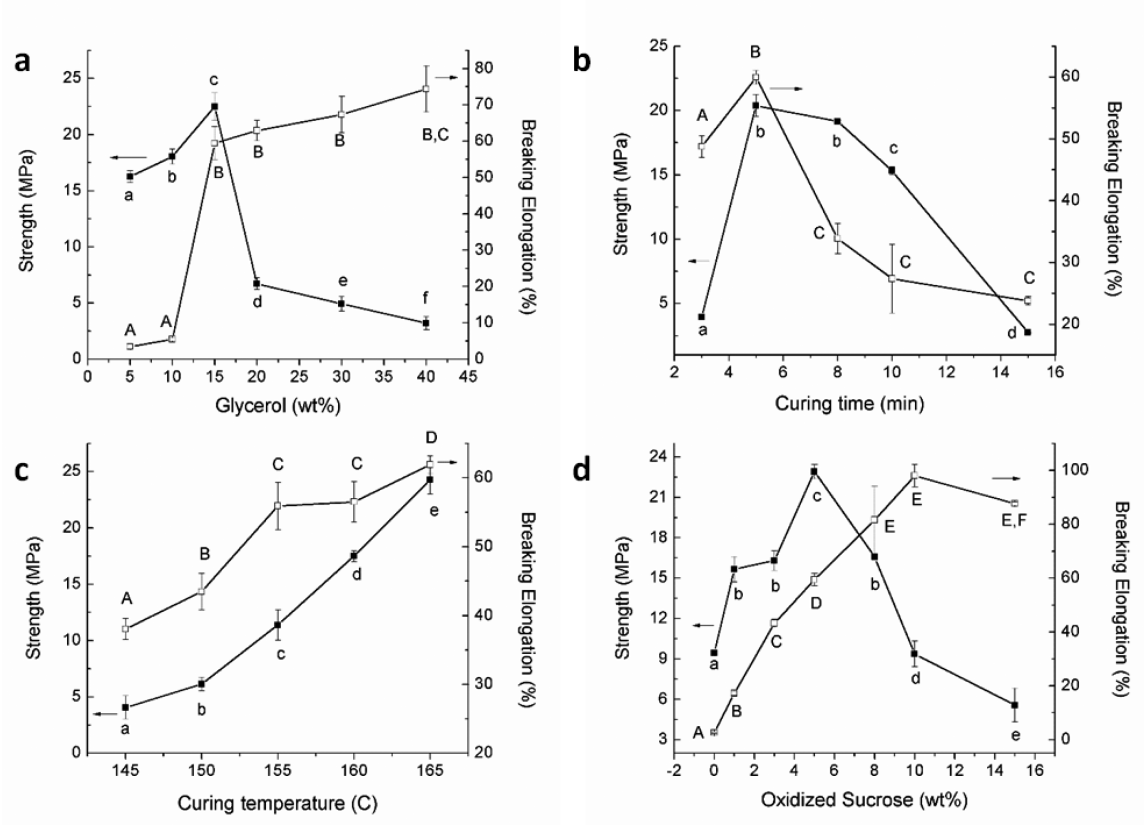


Figure 1. Effects of (a) weight percent of glycerol over starch (5 wt % oxidized sucrose, 165 °C, 5 min); (b) curing time (5 wt % oxidized sucrose, 15 wt % glycerol, 165 °C); (c) curing temperature (5 wt % oxidized sucrose, 15 wt % glycerol, 5 min); (d) weight percent of oxidized sucrose concentration (15 wt % glycerol, 165 °C, 5 min) on strength and elongation of cross-linked starch films. All the percentage was based on the weight of starch. Different letters indicated statistically significant difference among data points.

starch films, dynamic mechanical analyses on the starch films (length × width × thickness = 8.5 × 2.5 × 0.3 mm) were performed using a TA Instruments Q 800 (New Castle, DE) with tensioning cantilever geometry. Measurements were performed from 35 to 200 °C at a heating rate of 5 °C min^{-1}, strain of 0.1%, amplitude of 15.00 μm, and a frequency of 0.5 Hz. Storage modulus and tan δ of starch films before and after cross-linking were recorded.

Stability in Water. About 0.3 g of control and cross-linked starch films were soaked in 100 mL distilled water under room temperature for 3, 5, 10, 20, and 35 days. Temperature effect on water stability was also studied. About 0.3 g control and cross-linked films were weighed and put in 100 mL of distilled water under different temperatures (50, 60, 70, 80, and 90 °C) for 30 min. After soaked for designated time periods, the samples were taken out, rinsed with distilled water, and allowed to dry at 23 ± 0.5 °C and 50% RH in the conditioning chamber and weighed. Three replications were done for each sample.

The % weight loss was calculated according to eq 1.

$$\%\text{weight loss} = \frac{\text{initial weight of starch film} - \text{weight of starch film after incubation}}{\text{initial weight of starch film}} \quad (1)$$

Stability in Formic Acid. About 0.2 g of control and cross-linked starch films were put in 5 g formic acid, and the mixture was sealed tightly. They were kept at room temperature for 24 h. After treatment, formic acid was removed, and the remaining starch films were washed with distilled water repeatedly to neutral. The samples were dried at 23 ± 0.5 °C and 50% RH in the conditioning chamber and weighed. Three replications were done for each film. The % weight loss was calculated according to eq 1.

Thermal Analysis. Differential scanning calorimeter (DSC 822: Mettler-Toledo Inc., Columbus, OH) was used to study the thermal behavior of starch films before and after cross-linking. DSC curves were obtained by heating the samples at a rate of 20 °C per min, holding at 50 °C for 3 min to evaporate moisture, and again heating to 250 °C at 20 °C per minute. The samples were cooled to 25 °C at 20 °C per minute and a second heating cycle was obtained using the same procedure.

Reaction Kinetics. Kinetic study was conducted to determine rate law of oxidized sucrose cross-linking of starch. After determining rate order and rate constant (k), Arrhenius constant (A), and activation energy (Ea) were calculated according to the Arrhenius Equation, as shown in eq 2.

$$k = Ae^{-\mathrm{Ea}/RT} \quad (2)$$

where, R is the gas constant, 8.3145 J mol^{-1} K^{-1} and T is the curing temperature (K).

Because reaction between aldehyde groups of oxidized sucrose and hydroxyl groups of starch could introduce either intermolecular or intramolecular cross-links, the exact amount of cross-linking was almost impossible to measure. Thus, the amount of cross-links formed as a result of the cross-linking reaction was calculated based on the change of total amount of aldehyde groups introduced into the aqueous starch solution. About 0.5 g un-cross-linked and cross-linked films, which were cured at 140, 150, and 165 °C for 0, 3, 5, 10, and 15 min were put into 100 mL of distilled water. After 10 h, the films were removed and the filtrate with unreacted oxidized sucrose were collected. Aldehyde concentrations in the filtrate were quantified using iodometric titration. The unreacted oxidized sucrose concentration was subtracted from the total oxidized sucrose initially introduced into the starch solution and plotted against the curing time to obtain the reaction rate and reaction rate constant.

As the oxidized sucrose might self-cross-link due to heating, the same amount of oxidized sucrose powder obtained via low

DOI: 10.1021/acssuschemeng.5b00353
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temperature rotary evaporation was treated under the same conditions. The decrements in aldehyde groups after the treatments were considered as the amounts of aldehyde groups consumed due to self-cross-linking in the process of cross-linking, and were deduced from consumption of total aldehyde groups in the calculation as blanks.

Iodometric titration method was developed to determine the concentration of aldehyde groups. Excess amount (25 mL) of 0.1 M I_2/KI solution and 10 mL of 1 M NaOH solution were added slowly into the obtained filtrate. The mixture was kept in dark for 20 min to complete the reaction between iodine and aldehyde groups as shown in eqs 3 and 4. Because the final solution of oxidized sucrose was a mixture of different polyaldehyde derivatives, the reaction ratio between triiodine and aldehyde was assumed 1:1 as shown in eq 4. After 20 min, 15 mL of 0.5 M H_2SO_4 was added into the mixture in a controlled manner to adjust pH and to prevent the formation of sodium iodate. The mixture was titrated with standardized 0.1 M sodium thiosulfate solution. The aldehyde concentration was calculated based on the consumed thiosulfate volume as shown in eq 5.

$$I_2 + KI \rightarrow I_3^- \quad (3)$$

$$RC{-}OH\ (\text{aldehyde}) + I_3^- + NaOH \rightarrow RC{-}ONa \quad (4)$$

$$I_{3\text{excess}}^- + 2S_2O_3^{2-} \rightarrow S_4O_6^{2-} + 3I^- \quad (5)$$

A factor of 4 was considered in the calculation of concentration of oxidized sucrose in the kinetic study.

Statistics. One way analysis of variance was employed to analyze the data. The sample sizes were equal for each test, the observations being tested were independent within and among the groups, which were normally distributed and variances were homogeneous. Tukey's pairwise multiple comparison was chosen. The confidence interval was set at 95% and a p value less than 0.05 was considered to be a statistically significant difference. The data in the figures labeled with different numbers or characters indicated significant differences.

RESULTS AND DISCUSSION

Mechanical Properties of Films Cross-Linked under Different Conditions. Figure 1a illustrates that as glycerol concentration increased, the tensile strength of starch film increased and then decreased, whereas the elongation increased fast before the concentration reached 15% and slowly afterward. Plasticization was an unavoidable procedure to endow starch films with flexibility because both cross-linked and un-cross-linked starch films were too brittle. Glycerol was the most widely used plasticizer for biobased products.[25] The slight increase in tensile strength at low glycerol concentration might be due to improved capability for the starch molecules to align during stretching, which also caused significant increase in elongation of starch films. However, as the concentration of glycerol further increased, the molecular interaction decreased so remarkably that the molecules could not undertake load before they slid away from each other. The optimal concentration was 15 wt % as indicated by the highest tensile strength and relatively high elongation.

Figure 1b demonstrates that both tensile strength and elongation of starch films increased first and then decreased as cross-linking time increased. Curing time shorter than 5 min was insufficient to build a network among starch molecules, leading to weak intermolecular interaction. Curing for 5 min could enable oxidized sucrose to cross-link adequately starch and induce network formation among molecules, leading to the highest tensile strength and strain consequently. Further extending the curing time might damage starch backbones and also increased cross-linking density, and thus resulted in decrease in tensile properties.

Figure 1c demonstrates that both tensile strength and elongation of starch films gradually increased as cross-linking temperature increased from 145 to 165 °C. The temperature for the reaction should be controlled high enough to ensure efficient removal of water molecules from the reaction between OS and starch. At the same time, it could not be too high to minimize thermal decomposition of starch.[26] The temperature of 165 °C might be the optimal temperature for oxidized sucrose cross-linking.

Figure 1d demonstrates that both tensile strength and elongation of starch films increased first and then decreased as concentrations of oxidized sucrose increased. The highest tensile strength of 23 MPa and highest tensile strain of 95% were achieved as the concentration of oxidized sucrose were 5 and 10 wt %, respectively. Cross-linking built inter- and intramolecular linkages as well as lengthened the starch molecules. Subsequently, molecular interaction could be enhanced, resulting in increase in tensile strength and decrease in tensile strain. Meanwhile, when the cross-linking degree was low, the length of starch molecules available for sliding could be increased. However, continuous increase in cross-linking extent restricted the sliding of starch molecules due to increased inter- and intramolecular interaction, and thus decreased the tensile strain. Thus, breakage of weak points among the overcross-linked molecular sections became the major cause of film breakage, resulting in reduced tensile strength. The oxidized sucrose concentration of 5 wt % yielding best tensile strength and relative good tensile strain was used in later work.

As shown in Table 1, oxidized sucrose endowed the solvent-cast starch films with good tensile strength and strain simultaneously, whereas many other cross-linkers did not. For example, cross-linking using nontoxic citric acid or toxic epichlorohydrin could yield similar tensile strength, but much lower tensile strain; boric acid cross-linking improved tensile strength and strain at the same time, but the improvement was limited. The better improvement in mechanical properties could be attributed to higher activity of aldehyde groups in oxidized sucrose, compared with those in other cross-linkers.

Table 1. Comparison of Tensile Properties of Starch Films Treated with Different Cross-Linkers

cross-linking Agent	tensile strength (MPa)	tensile strain (%)	ref
un-cross-linked 15% glycerol		2.6	this work
oxidized sucrose	23	60	this work
citric acid	24	27	16
epichlorohydrin	50	5	27
epichlorohydrin (PE blended)	15.5	217	28
glutaraldehyde	0.38	10	29
boric acid (PVA blended)	12.15	15	30

Stability of Starch Films. *Thermal Stability.* Figure 2 shows the DSC thermograms of corn starch films before and after cross-linking with 5% oxidized sucrose, proving that cross-linking substantially enhanced thermal stability of starch films. A big and small first endothermic peak at around 160 °C existed in the thermograms of un-cross-linked and cross-linked starch, respectively. However, the un-cross-linked starch had a second endothermic peak lower than 170 °C, whereas the

DOI: 10.1021/acssuschemeng.5b00353
ACS Sustainable Chem. Eng. 2015, 3, 2631−2639

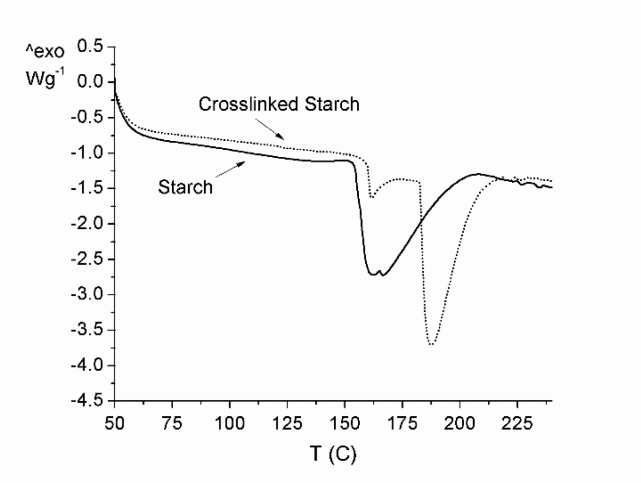

Figure 2. DSC thermograms of non-cross-linked (15 wt % glycerol) and cross-linked (5% oxidized sucrose, 15 wt % glycerol, 165 °C, 5 min) corn starch films.

cross-linked starch showed a second peak at around 188 °C. The first peak around 160 °C might be due to decomposition of starch molecules. For the thermogram of non-cross-linked starch, the melting peak was lost in broad decomposition peak.[31] The results were in accordance with the results reported by Liu et al.[32] and Kumar et al.[33] On the other hand, after cross-linking, the much higher decomposition temperature could be due to the more compact molecular structure and stronger intermolecular forces attributed to additional intra- and intermolecular bonds formed due to cross-linking.

Stability in Water. Figure 3 indicates that the oxidized sucrose cross-linked starch films showed exceptionally better water stability compared with the un-cross-linked ones. In Figure 3a, the cross-linked starch films retained about 80% initial weight whereas the un-cross-linked ones retained about 20% initial weight after incubated in water for up to 35 days. When the the incubation temperature was increased from 50 to 90 °C, cross-linked starch films preserved about 75% of its original weight, whereas the un-cross-linked counterpart dissociated immediately after soaked in water at 50 °C, as shown in Figure 3b. Cross-linking introduced new inter- and intramolecular covalent bonds, and thus the whole structures became less susceptible to water.

Stability in Formic Acid. Figure 4 demonstrates that weight loss of starch films in formic acid decreased significantly

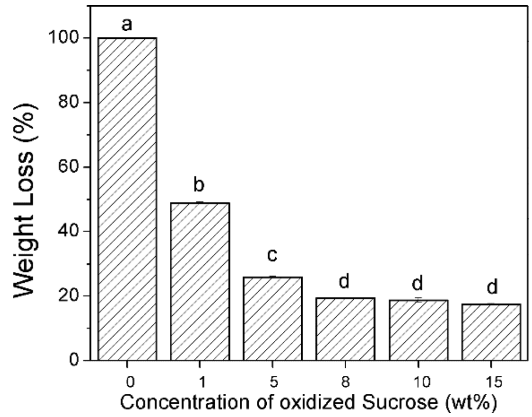

Figure 4. Weight loss of non-cross-linked (15 wt % glycerol) and cross-linked (0, 1%, 5%, 8%, 10%, and 15% oxidized sucrose, 15 wt % glycerol, 165 °C, 5 min) starch films at 25 °C in formic acid for 24 h. The weight ratio of solid to liquid was 1:50. Different letters indicate statistically significant differences among data points.

after cross-linking. Non-cross-linked films totally dissolved after being in formic acid for 24 h as expected. Films cross-linked with 1 wt % of oxidized sucrose lost had 49% of initial weight, whereas those cross-linked with 5, 8, 10, and 15 wt % oxidized sucrose showed weight loss of 26%, 19%, 19%, and 18%, respectively. Starch dissolved in formic acid due to breakage of inter- and intramolecular hydrogen bonds. Cross-linking created three-dimensional polymeric structures, which were stabilized via both hydrogen bonding and newly generated hemiacetal/acetal linkages, which might mainly contribute to the insolubility or slow solubility of cross-linked starch in formic acid.

Confirmation of Reaction between Oxidized Sucrose and Starch via FTIR and ^{1}H NMR. *FTIR of Oxidized Sucrose.* Figure 5a shows FTIR spectra of sucrose and oxidized sucrose. A peak at around 1650 cm^{-1} indicating the presence of aldehyde groups existed in the spectra of oxidized sucrose other than sucrose. In addition, a peak at around 3400 cm^{-1}

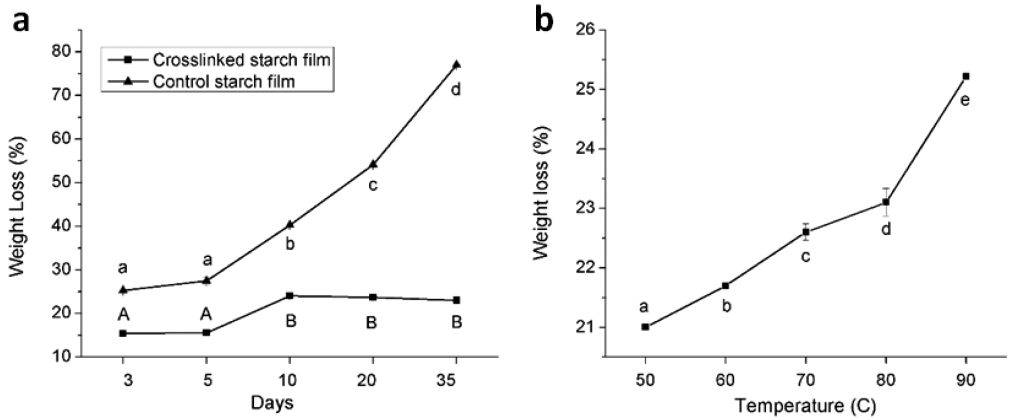

Figure 3. Weight loss of non-cross-linked (15 wt % glycerol) and cross-linked starch films (5% oxidized sucrose, 15 wt % glycerol, 165 °C, 5 min) under conditions (a). 25 °C in distilled water for 3, 5, 10, 20, and 35 days; (b). Distilled water at temperatures of 50, 60, 70, 80, and 90 °C for 30 min. The weight ratio of solid to liquid was 1:50. Different letters indicated statistically significant difference among data points.

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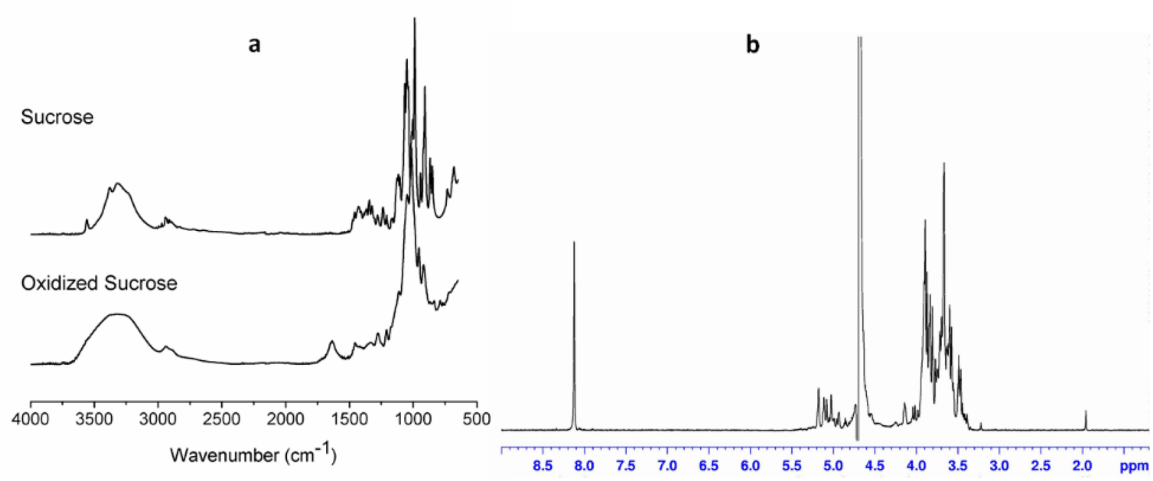

Figure 5. (a) FTIR spectra of sucrose and oxidized sucrose; (b) ^{1}H NMR spectra of oxidized sucrose.

representing hydroxyl group was much broader in the spectra of oxidized sucrose than in that of sucrose. Sucrose before and after cross-linking had unique combination of peaks in the fingerprint region from 600 to 900 cm^{-1}, due to C−O stretching of glycosidic bonds. In Figure 5b, the ^{1}H NMR spectra showed one proposed structure of hemiacetal derivatives of oxidized sucrose. The free aldehyde at 8−8.5 ppm and hemiacetal groups between 4.5 and 5 ppm proved that polyaldehyde derivatives were produced via oxidation of sucrose.

^{1}H NMR of Sucrose, Oxidized Sucrose, Starch, and Cross-Linked Starch. Figure 6 shows ^{1}H NMR spectra of un-cross-linked and cross-linked starch films. Splitting of peaks and a single peak at around 4.6 ppm in the spectra of cross-linked starch (Figure 6b) and un-cross-linked starch (Figure 6a), respectively. The splitting could be due to the acetal bonds formed between C-6 of starch and aldehyde groups of oxidized sucrose. Both spectra had chemical shifts at around 5.1 ppm, indicating the hydrogen bonded hydroxyl groups, which were abundant in both cross-linked and un-cross-linked starch.

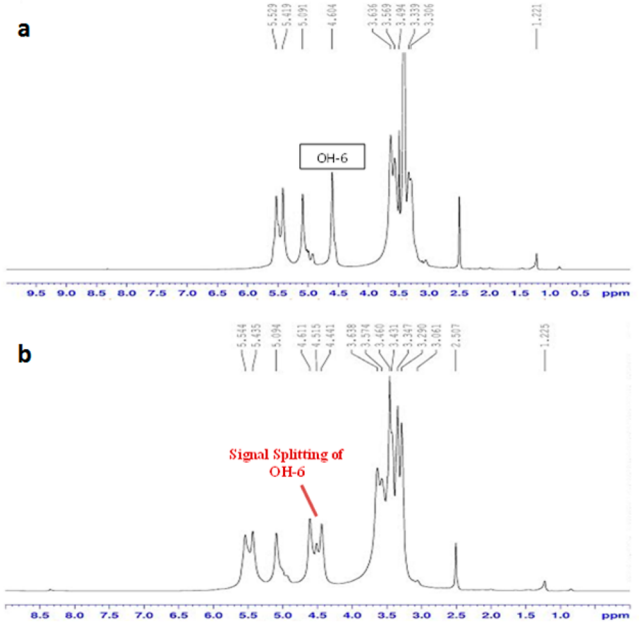

Figure 6. (a) Un-cross-linked starch film (15 wt % glycerol); (b) cross-linked starch film (cross-linking condition: 5 wt % oxidized sucrose, cured at 165 °C for 5 min).

Figure 7a shows the change of storage modulus and tan δ of starch films before and after cross-linking. Over the whole temperature range, cross-linked starch films had much higher storage modulus than the un-cross-linked ones, indicating substantially increased inherent stiffness of the starch films after cross-linking. In Figure 7b, the tan δ curve showed a peak at around 130 °C, indicating glass transition behavior of un-cross-linked starch films, whereas the other tan δ curve had a subtle peak at around 130 °C and an obvious peak at around 175 °C. The former peak might be attributed to the un-cross-linked starch molecules whereas the latter peak might be due to cross-linked starch network. The phenomenon might be attributed to an incomplete formation of cross-linked starch network.

Kinetics of Cross-Linking Reaction. Figure 8 shows change of concentrations of oxidized sucrose ([OS]) as reaction time prolonged under different temperatures. As the reactions approached equilibrium, the concentrations of OS decreased to constant values. More OS was consumed in reactions under higher temperature. In this study, excessive starch was used and the temperature was high enough to evaporate water, one of the products, and thus the reaction could be considered as irreversible. Therefore, the reaction rate r was calculated according to eq 6.

$$r = -\frac{\mathrm{d[OS]}}{\mathrm{d}t} = -\frac{\mathrm{d[St]}}{\mathrm{d}t} = -k[\mathrm{OS}]^{a}[\mathrm{St}]^{b} \quad (6)$$

wherein [OS] is the oxidized sucrose concentration, [St] is the starch concentration, r is the reaction rate, k is the rate constant, and a and b are the orders of reactions.

Here, the concentration of starch was considered as constant, since excessively amount was added into the system. Thus, it was expected that the reaction of aldehyde sides of oxidized sucrose with these hydroxyl groups follows pseudo-first-order kinetics.[34] Thus, in the pseudo-first-order rate equation, a will be 1, whereas b will be 0 based on the assumption made above. The pseudo-first-order reaction rate was found as shown in eq 7, where k' is the new pseudo-first-order rate constant.

$$r = -\frac{\mathrm{d[OS]}}{\mathrm{d}t} = -k'[\mathrm{OS}]^{a} \quad (7)$$

DOI: 10.1021/acssuschemeng.5b00353
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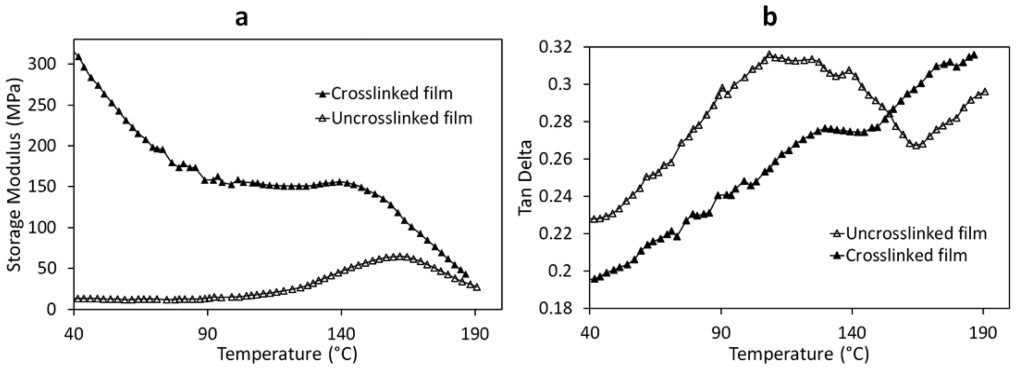

Figure 7. (a) Storage modulus and (b) tan δ of cross-linked and un-cross-linked starch films (cross-linking condition: 5 wt % oxidized sucrose, cured at 165 °C for 5 min).

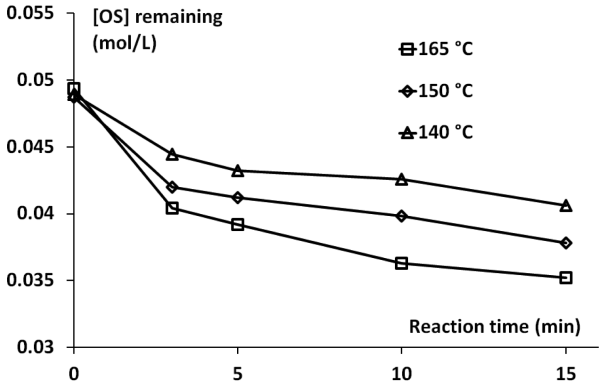

Figure 8. Change of concentration of oxidized sucrose after reacted with starch at 140, 150, and 165 °C for 0−15 min. Decrease of the concentration of oxidized sucrose in the titration solution as a function of curing time for different curing temperatures (140, 150, and 165 °C). All films contained 5 wt % oxidized sucrose.

After integration of the rate equation above, the correlation between oxidized sucrose and curing time was found as shown in eq 8, where $[OS]_0$ indicates the concentration of oxidized sucrose at t = 0 min.

$$\ln[OS]_0 - \ln[OS] = k't \quad (8)$$

According to the eq 8, the rate constant k' can be determined from the value of the slope of the plot ln $[OS]_0$ − ln [OS] against the curing time t, and the regression lines are shown in Figure 9a.

After the rate constants were determined for different curing temperatures, E_a was calculated from the slope of the plot ln k against $1/T$ and A was calculated from the intercept of the plot ln k against $1/T$ based on eq 2, and the regression line can be seen in Figure 9b.

All these kinetic parameters are shown in Table 2. As it was expected, rate constant increased with curing temperature and the highest rate constant, 11.8 × 10^{-3} s^{-1}, was obtained as a result of curing at higher temperature, 165 °C. According to the Arrhenius equation stated in eq 2, rate constant and temperature has a negative correlation. On the molecular level, increasing curing temperature allows molecules to move faster and thus enhances the chance of reaction between aldehyde groups of oxidized sucrose and hydroxyl groups of starch. Additionally, the Arrhenius constant was found as 107.48 s^{-1}.

Table 2. Kinetic Data of the Crosslinking Reaction of 5% Oxidized Sucrose with Corn Starch at Different Curing Temperatures

k (s^{-1} × 10^{-3})			E_a (kJ mol^{-1})	A (s^{-1})
140 °C	150 °C	165 °C		
6.8	8.5	11.8	33.22	107.48

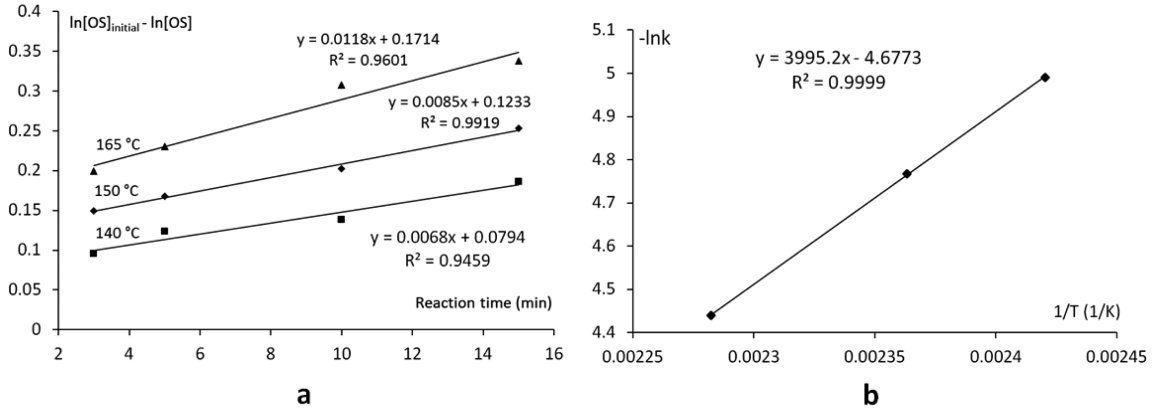

Figure 9. (a) Regression lines of ln $[OS]_0$ − ln [OS] as a function of time to determine the reaction constants of oxidized sucrose at different curing temperatures (140, 150, and 165 °C); (b) regression line to determine the Arrhenius constant, A and activation energy, E_a of corn starch cross-linking with 5 wt % oxidized sucrose.

DOI: 10.1021/acssuschemeng.5b00353
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On the other hand, the activation energy of the reaction, which indicates the minimum energy required to start cross-linking formation, was found 33.22 kJ mol^{-1}. Cross-linking between aldehyde groups of oxidized sucrose and hydroxyl groups of starch could occur when the energy in the system is only 33.22 kJ mol^{-1}, which is significantly lower than the activation energies of reaction between many current cross-linking agents and polysaccharides. The comparison of oxidized sucrose with the current cross-linking agents can be seen in Table 3 in terms of activation energy.

Table 3. Comparison of Activation Energy for Starch Crosslinking Using Different Cross-Linkers

source	cross-linking agent	E_a (kJ mol^{-1})	ref
corn	oxidized sucrose	33.22	this work
corn	ammonium zirconium carbonate	139	35
corn	epichlorohydrin	38	36
cassava	tetraethylene glycol diacrylate	240	37
potato	1,2,3,4-diepoxybutane	55	38
potato	1,2,7,8-diepoxyoctane	105	38

As shown in Table 3, the lowest activation energy for starch cross-linking was obtained by oxidized sucrose, which was 33.22 kJ mol^{-1}. Epichlorohydrin was the second lowest with activation energy of 38 kJ mol^{-1}. However, epichlorohydrin is classified as a human carcinogen as stated earlier.[13] Additionally, previous researches resulted that DEB cross-linking requires 55 kJ mol^{-1} for potato starch. Rather than these two cross-linkers, all the other agents have either high activation energies or not been studied yet. The lowest activation energy, inferring that the reaction occurred more readily between starch and oxidized sucrose than most currently existing cross-linkers.

CONCLUSIONS

In this study, oxidized sucrose, a nontraditional biobased polyaldehyde, was used to cross-link starch film. The obtained starch films were found strong and ductile with maximum tensile strength of 23 MPa and maximum strain of 100%. The films retained about 80% of their weight after soaked in water at room temperature for up to 35 days, or in water at 90 °C for 30 min, or in formic acid at room temperature for 24 h. The signal splitting of acetal carbon in ^{1}H NMR spectra verified cross-linking reaction between oxidized sucrose and starch. The cross-linking was a pseudo-first-order reaction with activation energy of 33.22 kJ mol^{-1}, lower than that of reactions between a variety of cross-linkers and starch. The cross-linking could occur easily. In general, oxidized sucrose could be a highly efficient biobased cross-linker to improve the performance properties of starch products.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was financially supported by Nebraska Corn Board, USDA - National Institute of Food and Agriculture (Hatch Act, Multistate Research Project S-1054 (NEB37-037)), and Agricultural Research Division at the University of Nebraska−Lincoln.

REFERENCES

(1) (a) Li, Z.; Wang, J.; Cheng, L.; Gu, Z.; Hong, Y.; Kowalczyk, A. Improving the performance of starch-based wood adhesive by using sodium dodecyl sulfate. *Carbohydr. Polym.* **2014**, *99*, 579−583. (b) Ibrahim, H.; Farag, M.; Megahed, H.; Mehanny, S. Characteristics of starch-based biodegradable composites reinforced with date palm and flax fibers. *Carbohydr. Polym.* **2014**, *101*, 11−19. (c) González-Gutiérrez, J.; Partal, P.; García-Morales, M.; Gallegos, C. Effect of processing on the viscoelastic, tensile and optical properties of albumen/starch-based bioplastics. *Carbohydr. Polym.* **2011**, *84* (1), 308−315.

(2) (a) van Soest, J. J.; Hulleman, S.; De Wit, D.; Vliegenthart, J. Crystallinity in starch bioplastics. *Ind. Crops Prod.* **1996**, *5* (1), 11−22. (b) Ghosh Dastidar, T.; Netravali, A. Cross-Linked Waxy Maize Starch-Based "Green" Composites. *ACS Sustainable Chem. Eng.* **2013**, *1* (12), 1537−1544.

(3) Arvanitoyannis, I.; Biliaderis, C. G.; Ogawa, H.; Kawasaki, N. Biodegradable films made from low-density polyethylene (LDPE), rice starch and potato starch for food packaging applications: Part 1. *Carbohydr. Polym.* **1998**, *36* (2), 89−104.

(4) Kim, S.; Adkins, J.; Aglan, H. A.; Biswas, A.; Selling, G. Polymer composites prepared from heat-treated starch and styrene-butadiene latex. *J. Elastomers Plast.* **2014**, 0095244314538440.

(5) Yoon, S.-D.; Park, M.-H.; Byun, H.-S. Mechanical and water barrier properties of starch/PVA composite films by adding nano-sized poly (methyl methacrylate-*co*-acrylamide) particles. *Carbohydr. Polym.* **2012**, *87* (1), 676−686.

(6) (a) Müller, C. M.; Laurindo, J. B.; Yamashita, F. Effect of nanoclay incorporation method on mechanical and water vapor barrier properties of starch-based films. *Ind. Crops Prod.* **2011**, *33* (3), 605−610. (b) Kaur, H.; Banipal, T. S.; Thakur, S.; Bakshi, M. S.; Kaur, G.; Singh, N. Novel Biodegradable Films with Extraordinary Tensile Strength and Flexibility Provided by Nanoparticles. *ACS Sustainable Chem. Eng.* **2012**, *1* (1), 127−136.

(7) Parra, D.; Tadini, C.; Ponce, P.; Lugão, A. Mechanical properties and water vapor transmission in some blends of cassava starch edible films. *Carbohydr. Polym.* **2004**, *58* (4), 475−481.

(8) Yang, Y.; Wang, L.; Li, S. Formaldehyde-free zein fiber—preparation and investigation. *J. Appl. Polym. Sci.* **1996**, *59* (3), 433−441.

(9) Kulkarni, A. R.; Soppimath, K. S.; Aminabhavi, T. M.; Dave, A. M.; Mehta, M. H. Urea−Formaldehyde crosslinked starch and Guar gum matrices for encapsulation of natural liquid pesticide [Azadirachta Indica A. Juss.(Neem) Seed oil]: swelling and Release kinetics. *J. Appl. Polym. Sci.* **1999**, *73* (12), 2437−2446.

(10) Han, X.; Chen, S.; Hu, X. Controlled-release fertilizer encapsulated by starch/polyvinyl alcohol coating. *Desalination* **2009**, *240* (1), 21−26.

(11) (a) Ma, T.-H.; Harris, M. M. Review of the genotoxicity of formaldehyde. *Mutat. Res., Rev. Genet. Toxicol.* **1988**, *196* (1), 37−59. (b) Di Stefano, F.; Siriruttanapruk, S.; McCoach, J.; Sherwood Burge, P. Glutaraldehyde: an occupational hazard in the hospital setting. *Allergy* **1999**, *54* (10), 1105−1109.

(12) Kuniak, L.; Marchessault, R. Study of the crosslinking reaction between epichlorohydrin and starch. *Starch-Stärke* **1972**, *24* (4), 110−116.

(13) Lynch, B. S.; Bryant, D. W.; Hook, G. J.; Nestmann, E. R.; Munro, I. C. Carcinogenicity of monochloro-1, 2-propanediol (α-chlorohydrin, 3-MCPD). *Int. J. Toxicol.* **1998**, *17* (1), 47−76.

(14) (a) Hwang, D. K.; Kim, B. Y.; Baik, M. Y. Physicochemical Properties of Non-thermally Cross-linked Corn Starch with Phosphorus Oxychloride using Ultra High Pressure (UHP). *Starch-Stärke* **2009**, *61* (8), 438−447. (b) Quistad, G. B.; Zhang, N.; Sparks, S. E.; Casida, J. E. Phosphoacetylcholinesterase: toxicity of phosphorus oxychloride to mammals and insects that can be attributed to selective

DOI: 10.1021/acssuschemeng.5b00353
ACS Sustainable Chem. Eng. 2015, 3, 2631−2639

phosphorylation of acetylcholinesterase by phosphorodichloridic acid. *Chem. Res. Toxicol.* **2000**, *13* (7), 652−657.
(15) (a) Cai, S.; Xu, H.; Jiang, Q.; Yang, Y. Novel 3D electrospun scaffolds with fibers oriented randomly and evenly in three dimensions to closely mimic the unique architectures of extracellular matrices in soft tissues: fabrication and mechanism study. *Langmuir* **2013**, *29* (7), 2311−2318. (b) Xu, H.; Shen, L.; Xu, L.; Yang, Y. Controlled delivery of hollow corn protein nanoparticles via non-toxic crosslinking: in vivo and drug loading study. *Biomed. Microdevices* **2015**, *17* (1), 1−8.
(16) Reddy, N.; Yang, Y. Citric acid cross-linking of starch films. *Food Chem.* **2010**, *118* (3), 702−711.
(17) (a) Delville, J.; Joly, C.; Dole, P.; Bliard, C. Solid state photocrosslinked starch based films: a new family of homogeneous modified starches. *Carbohydr. Polym.* **2002**, *49* (1), 71−81. (b) Delville, J.; Joly, C.; Dole, P.; Bliard, C. Influence of photocrosslinking on the retrogradation of wheat starch based films. *Carbohydr. Polym.* **2003**, *53* (4), 373−381.
(18) Wilson, R. H. Utilization and toxicity of dialdehyde-and dicarboxyl-starches. *Exp. Biol. Med.* **1959**, *102* (3), 735−737.
(19) Zhang, X.; Yang, Y.; Yao, J.; Shao, Z.; Chen, X. Strong Collagen Hydrogels by Oxidized Dextran Modification. *ACS Sustainable Chem. Eng.* **2014**, *2* (5), 1318−1324.
(20) Rhim, J.-W.; Gennadios, A.; Weller, C. L.; Carole, C.; Hanna, M. A. Soy protein isolate−dialdehyde starch films. *Ind. Crops Prod.* **1998**, *8* (3), 195−203.
(21) Gennadios, A.; Handa, A.; Froning, G. W.; Weller, C. L.; Hanna, M. A. Physical properties of egg white-dialdehyde starch films. *J. Agric. Food Chem.* **1998**, *46* (4), 1297−1302.
(22) Liu, L.; Chen, Z.; Wang, B.; Yang, Y. Improving wet strength of soy protein films using oxidized sucrose. *J. Appl. Polym. Sci.* **2015**, *132* (7), DOI: 10.1002/app.41473.
(23) (a) Xu, H.; Liu, P.; Mi, X.; Xu, L.; Yang, Y. Potent and regularizable crosslinking of ultrafine fibrous protein scaffolds for tissue engineering using a cytocompatible disaccharide derivative. *J. Mater. Chem. B* **2015**, *3*, 3609−3616. (b) Liu, P.; Xu, H.; Mi, X.; Xu, L.; Yang, Y. Oxidized Sucrose: A Potent and Biocompatible Crosslinker for Three-Dimensional Fibrous Protein Scaffolds. *Macromol. Mater. Eng.* **2015**, *300* (4), 414−422.
(24) Barry, V. C.; Mitchell, P. Properties of periodate-oxidised polysaccharides. Part IV. The products obtained on reaction with phenylhydrazine. *J. Chem. Soc.* **1954**, 4020−4023.
(25) McHugh, T. H.; Krochta, J. M. Sorbitol-vs glycerol-plasticized whey protein edible films: integrated oxygen permeability and tensile property evaluation. *J. Agric. Food Chem.* **1994**, *42* (4), 841−845.
(26) Curvelo, A.; De Carvalho, A.; Agnelli, J. Thermoplastic starch−cellulosic fibers composites: preliminary results. *Carbohydr. Polym.* **2001**, *45* (2), 183−188.
(27) Rioux, B.; Ispas-Szabo, P.; Aït-Kadi, A.; Mateescu, M.-A.; Juhász, J. Structure−properties relationship in cross-linked high amylose starch cast films. *Carbohydr. Polym.* **2002**, *50* (4), 371−378.
(28) Kim, M.; Lee, S.-J. Characteristics of crosslinked potato starch and starch-filled linear low-density polyethylene films. *Carbohydr. Polym.* **2002**, *50* (4), 331−337.
(29) Parra, D. F.; Tadini, C. C.; Ponce, P.; Lugão, A. B. Mechanical properties and water vapor transmission in some blends of cassava starch edible films. *Carbohydr. Polym.* **2004**, *58* (4), 475−481.
(30) Yin, Y.; Li, J.; Liu, Y.; Li, Z. Starch crosslinked with poly(vinyl alcohol) by boric acid. *J. Appl. Polym. Sci.* **2005**, *96* (4), 1394−1397.
(31) Van Soest, J.; Borger, D. Structure and properties of compression-molded thermoplastic starch materials from normal and high-amylose maize starches. *J. Appl. Polym. Sci.* **1997**, *64* (4), 631−644.
(32) Liu, Z. Q.; Yi, X.-S.; Feng, Y. Effects of glycerin and glycerol monostearate on performance of thermoplastic starch. *J. Mater. Sci.* **2001**, *36* (7), 1809−1815.
(33) Kumar, A. P.; Singh, R. P. Biocomposites of cellulose reinforced starch: Improvement of properties by photo-induced crosslinking. *Bioresour. Technol.* **2008**, *99* (18), 8803−8809.
(34) (a) Birla, A.; Singh, B.; Upadhyay, S. N.; Sharma, Y. C. Kinetics studies of synthesis of biodiesel from waste frying oil using a heterogeneous catalyst derived from snail shell. *Bioresour. Technol.* **2012**, *106* (0), 95−100. (b) Zhang, L.; Sheng, B.; Xin, Z.; Liu, Q.; Sun, S. Kinetics of transesterification of palm oil and dimethyl carbonate for biodiesel production at the catalysis of heterogeneous base catalyst. *Bioresour. Technol.* **2010**, *101* (21), 8144−8150. (c) Lund, K.; Brelid, H. Kinetics of cross-linking softwood kraft pulp with 1, 2, 3, 4-butanetetracarboxylic acid. *Ind. Eng. Chem. Res.* **2013**, *52* (33), 11502−11509.
(35) Song, D.; Breedveld, V.; Deng, Y. Rheological study of self-crosslinking and co-crosslinking of ammonium zirconium carbonate and starch in aqueous solutions. *J. Appl. Polym. Sci.* **2011**, *122* (2), 1019−1029.
(36) Dumitriu, S. Polysaccharides as biomaterials. In *Polymeric Biomaterials*; Marcel Dekker, Inc.: New York, 2001; pp 1−61.
(37) Marques, P.; Lima, A.; Bianco, G.; Laurindo, J.; Borsali, R.; Le Meins, J.-F.; Soldi, V. Thermal properties and stability of cassava starch films cross-linked with tetraethylene glycol diacrylate. *Polym. Degrad. Stab.* **2006**, *91* (4), 726−732.
(38) Šimkovic, I.; Hricovíni, M.; Mendichi, R.; van Soest, J. J. Cross-linking of starch with 1,2,3,4-diepoxybutane or 1,2,7,8-diepoxyoctane. *Carbohydr. Polym.* **2004**, *55* (3), 299−305.

DOI: 10.1021/acssuschemeng.5b00353
ACS Sustainable Chem. Eng. 2015, 3, 2631−2639